APPENDIX B

METHODS OF SCREENING FOR ANTIBODY LIGHT CHAINS

Technical Field

The present invention relates to methods of screening for high-affinity light chains which correspond to, and are commonly shared by, heavy chains having different specificities for a multi-specific antibody.

Background Art

Bispecific antibodies (BsAbs), also called bifunctional antibodies, are multivalent antibodies with specific binding sites for two antigenic determinants and which can react with two types of antigens. BsAbs can be produced using hybrid hybridomas, or more specifically, quadromas which are fusions of two different types of monoclonal antibody-producing cells (U.S. Patent No. 4,474,893; R. Bos and W. Nieuwenhuitzen (1992) Hybridoma 11(1): 41-51). BsAbs can also be generated by linking Fab (antigen-binding) fragments or Fab' fragments of two types of monoclonal antibodies, using chemical techniques (M. Brennan *et al.* (1985) Science 229(1708): 81-3) or by genetic engineering. In addition, BsAbs can be produced by covalently linking two complete monoclonal antibodies (B. Karpovsky *et al.* (1984) J. Exp. Med. 160(6): 1686-701).

Problems underlying BsAb production methods include the possibility of generating ten different types of antibody molecules due to random combination of immunoglobulin heavy chains and light chains (M.R. Suresh *et al.* (1986) Methods Enzymol. 121: 210-28). Among the ten types of antibodies produced by quadromas, the only antibody that has the desired dual specificity is the one that has the correct light and heavy chain combination and which is composed of two light chain/heavy chain pairs having different binding specificities. Therefore, the antibody having the desired dual specificity must be selectively purified from the ten types of antibodies produced by quadromas. Purification is generally performed using affinity chromatography, but this method is laborious and has low yields (Y.S. Massimo *et al.* (1997) J. Immunol. Methods 201: 57-66).

Methods that overcome such problems and give higher BsAb yields include, for example, methods of chemically linking antibody fragments such as Fab'-thionitrobenzoic acid derivative and Fab'-thiol (SH) (Brennan *et al.* (1985) Science 229: 81). Furthermore, methods for more conveniently obtaining chemically linkable Fab'-SH fragments include methods for producing these fragments from hosts such as *E. coli* using genetic recombination techniques (Shalaby *et al.* (1992) J. Exp. Med. 175: 217-25). Genetic recombination techniques can also be used to obtain BsAbs composed of humanized antibody fragments. Diabodies (Db) are BsAbs constructed from gene fusion of two types of fragments, and comprise a light chain